## Remarks

Applicants have reintroduced claim 13.

Applicants have submitted formal drawings and amended page 13 of the specification as required in the Office Action.

Applicants have amended claim 2 to insert the article "a" as required. Withdrawal of this Objection is requested.

The Office Action has rejected claims 1-6 and 13-16 for failing to comply with the enablement requirement. In making this rejection, the Examiner seems to be of the opinion that the state of the prior art and the level of one skilled in the art is much less developed than it really is. Many of the objections are outdated.

Submitted herewith as Appendices A and B are two documents which show the state of the art at the time the present application was filed. The first document is a re-print of the inventor's article on this subject which was published in *Expert Opinion on Biological Therapy* 2003; 3:215-226. This invited review is extensively referenced, and addresses many of the objections set forth in the Office Action. The second document is a brief list of early clinical trials recently undertaken in many countries which directly addresses Item 3 (the state of the art) which states that "the state of the art is nowhere near being able to use marrow stem cells in any reasonably predictable manner to improve cardiac function or treat heart failure in general." In view of these trials, which are extensively referenced elsewhere, one of skill in the art would know how to make and/or use the invention for improving cardiac function or treating cardiac failure. The Office Action expresses doubt that marrow stromal cells would differentiate into cardiomyocytes and become a living part of the myocardial tissue effected, as stated in the application. In this regard, please see the article identified by the reference number 62 (Chemdrawy EG et al., J. Thorac. Cardiovasc. Surg. 2002; 124:584-590) from the inventor's enclosed article. The fact that such cell transplant therapy could improve cardiac function has been confirmed by many authors, including Orlic D et al in *Nature in* 2001 (see reference number 25 in the enclosed article).

In summary, these two documents establish that the state of the art and the level of one skilled in the art is much more advanced that what the Examiner alleges, and that the artisan would have no difficulty in reproducing the invention based on the amount of guidance provided in the application. Thus, the claimed invention is enabled.

While, the Office Action alleges that the present application encompasses a wide area of knowledge, this should not prevent Applicant from obtaining a patent on the grounds of enablement.

In determining the nature of the invention, the Office Action relies on the prior art of Li et al. to show that the injection of fetal cardiomyocytes through differences in developed pressure can improve heart function. However, the Office Action also states that these fetal cardiomyocytes are not MSCs. In view of the enclosed articles, the use of MSCs is much more advanced than the Office Action states, and the same teaching that relates to fetal cardiomyocytes can be applied to MSCs. The Office Action also refers to Makino et al. to show that MSCs can be used to form cells with characteristics similar to fetal ventricular cardiomyocytes, but mentions that this article uses a cell line, which is indeed different from cells which have not been immortalized, as in the present invention. However, the inventors have demonstrated in the present invention that, with Figs. 2A and 2B, the method of the present invention can be used for improving cardiac function, regardless of whether the MSCs have been immortalized. This is somewhat contradictory to the assertion in the Office Action. The numerous clinical trials that are currently being undertaken in many countries clearly indicate an expectation of eventual success.

Withdrawal of the rejection under 35 U.S.C. §112 for lack of enablement is requested in view of the above remarks and enclosed materials.

Should there be any additional fees required, the Commissioner is hereby authorized to charge Deposit Account No. 23-0785.

Respectfully submitted,

WOOD, PHILLIPS, KATZ, CLARK & MORTIMER

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I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on July 26, 2004 and is addressed to Mail Stop Patent Application, Commissioner for Patents, P. O. Box 1450, Alexandria, Virginia 22313-1450.

Rebecca J. Willis

Expert Opinion



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Cell- & Tissue-based Therapy

# Adult stem cell therapy for heart failure

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Evidence indicates that bone marrow and many other somatic tissues contain pluripotent or multipotent adult stem cells as well as progenitor cells which can differentiate into cells of various phenotypes. Experimental studies strongly suggest that the normal function of the marrow derived adult stem cells is for tissue repair, and that they can be recruited by signals originating from injured tissue, traffic through the circulation and home into the injured site to undergo *milieu* dependent differentiation *in situ*. In the heart, these cells may differentiate into cardiomyocytes, vascular cells and scar tissue, thus participating in vasculogenesis, scar maturation and modulation of the remodelling process of the myocardium. To augment such a healing process, cell therapy using such cells, which may be preprogrammed if desired, may have donor cells implanted by direct injection, coronary infusion and, in some cases, by systemic intravenous administration. Improved ventricular function has been reported in myocardial infarct animal models. Although early Phase I clinical trials have been initiated for both autologous myoblast and autologous marrow cell transplants with favourable reported outcomes, the data are still too preliminary to draw definitive conclusions regarding their safety and efficacy. Additional mechanistic and translational preclinical investigations are essential, and well designed clinical studies are required before the great potential of adult stem cell therapy can be fully realised and benefit the vast number of heart failure patients.

Keywords: adult stem cells, bone marrow cells, heart failure therapy, myoblasts, myocardial infarction

*Expert Opin. Biol. Ther. (2003) 3(2):215-225*

## 1. Introduction

Stem cell therapy for tissue regeneration and repair is receiving intense interest from clinicians, academic scientists and the biotechnology sector, as well as the mass media, patients and general population. The potential of using stem cells to cure currently untreatable diseases is vast, and breathtaking progress is currently being made. Using adult stem cells as the donor source for such a therapy avoids the moral, religious and political controversies associated with the use of embryonic stem cells, which centre on the issues of obtaining such cells from human embryos as well as the use of cloning technology to avoid allograft rejection. In this overview, the goal is not to undertake an extensive review of mechanistic cellular and molecular studies, but rather to focus on two aspects: first, to examine issues and questions relevant to the clinical application of this approach; and second, to provide the most updated information available at the time of this writing. For the latter, the author will rely heavily on a large number of papers presented at the Annual Scientific meeting of the American Heart Association, which took place on 17 – 20 November 2002 in Chicago. Many of these presentations will be cited as abstracts, since it will take many months for them to go through the peer review process and be

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published as full papers. Thus, some of the data presented here should be seen as the cutting edge trend in research ongoing in this field, rather than as definitive rigorously peer reviewed scientific findings. Without taking some such risks in this rapidly progressing field, the information and the author's opinion expressed in this article could become outdated by the time this review is published.

## 2. The definition, origin and variety of 'adult stem cells'

### 2.1 Adult versus embryonic stem cells

Although all the somatic cells in the body originated from embryonic stem cells, it is not clear at present whether any of the pluripotent embryonic stem cells persisted until adult life to become adult stem cells. Likewise, the relationships among haematopoietic stem cells, the marrow stroma-derived 'mesenchymal stem cells' [1] and the resident stem cells found in various tissues such as the skin [2], liver [3] and brain [4], still are not clear. Although both embryonic stem cells and adult stem cells are pluripotent and capable of differentiating into various phenotypes, they appear to have different biological capabilities. A detailed discussion of the definition [5] and various characteristics of embryonic stem cells [6,7] is outside of the scope of this overview on adult stem cell therapy, except to suggest that embryonic stem cells, as they differentiate into various somatic cells, appear to be capable of following a supra-cellular structural design to create anatomical scaffolds and form various organs [8], while the adult stem cells can only differentiate into cells which may be integrated into the pre-existing anatomical structures. Whether such differences are due to intrinsic differences in these two types of stem cell or are due to their different microenvironments, also needs to be studied.

These differences between embryonic and adult stem cells reflect their apparently different biological roles. In contrast to the embryonic stem cells, whose goal is to develop a new organism, cumulative information gathered during the past decade suggests that adult stem cells may participate in tissue growth and repair. In studies on fetus [9] and postnatal animals [10] in which marrow stroma-derived mesenchymal stem cells were systematically administered, these cells were found to engraft in the tissues of various organs and undertook *in situ* differentiation, expressing phenotypes similar to their neighbouring cells. In the fully grown organisms, resident stem cells, such as those in the intestinal mucosal crypts [11], may have a primary role in cell turnover, replacing cells lost by apoptosis or mechanical attrition. However, there is increasing evidence that the adult stem cells residing in the bone marrow serve primarily as reserves for tissue repair [12,13].

### 2.2 Defining 'adult stem cells'

Two major features of adult stem cells are their capabilities for self-renewal and multilineage differentiation. Recently, the nature of the observed plasticity of adult stem cells had been challenged [14,15], as these cells were shown to be able to express different phenotypes by cell–cell fusions. The notion that such a finding denies the existence of multipotential adult stem cells has been refuted by subsequent studies, such as those reported by Verfaillie's group [16], which demonstrated that a single adult stem cell derived from the bone marrow could differentiate into various phenotypes in the absence of any differentiated cells for them to fuse with. This and other controversies [17] on adult stem cell plasticity reflect our limited knowledge at this stage of stem cell biology.

At the molecular level, there persists considerable confusion as to what one calls 'adult stem cells' and the progenitor cells which may be multipotent prior to their commitment to a specific lineage. Various investigators employ different molecular cell markers, using different culture conditions and cell separation techniques, all of which make it difficult to compare data from different laboratories [18]. For example, some investigators use Caplan's method [19], harvesting bone marrow cells which adhere to the bottom of tissue culture plates and calling them 'marrow stromal cells' [20,21]. While multipotent adult stem cells are present among marrow stromal cells, not all marrow stromal cells are multipotential [22]. Since *in vitro* culture conditions can alter a cell's proliferation and differentiation potentials, characterisation of their identity often depends on the molecular markers chosen. Thus, while Pittinger *et al.* [23,24] used the clonal homogeneity and the capacity for multipotential mesenchymal differentiation to define their adult stem cells, others used combinations of various molecular cell markers to define their bone marrow-derived stem cells. Thus, the cells of Orlic *et al.* [25] were $Lin^-$ $c\text{-}kit^{pos}$; Lichtenberg *et al.* [26], Sca-1 + $Lin^-$/$c\text{-}kit^+$ cells; Mangi *et al.* [27], c-kit+CD34- cells; Saw *et al.* [28] used 'Lentiviral transduced side population (SP) cells'; and Galinanes *et al.* [29] used CD34/CD117 dual positive cells for their clinical myocardial implantation. Jiang *et al.* [30] recently characterised their multipotent adult progenitor cells (MAPCs) to be: 'CD34, CD44, CD45, c-Kit and major histocompatibility complex (MHC) Class I and II negatives, expressing low levels of Flk-1, Sca-1 and Thy-1 and higher levels of CD13 and stage-specific embryonic antigen-1 (SSEA-1)'. Because of such differences in the definition of 'adult stem cells', it is often difficult to compare the findings from one group of investigators with those of others. In a recent workshop on cell therapy, sponsored by the National Institutes of Health in the US, there was a consensus that further investigation to enable standardisation of such definitions and classifications should be a high priority for scientists working in this area.

### 2.3 Adult skeletal myoblasts as donor cells

Skeletal muscle of humans and mammalian animals contains 'satellite cells' which lie adjacent to the skeletal myofibre, beneath the basement membrane but outside of the sarcolemma. These satellite cells are now known to be myoblasts, which are skeletal muscle progenitor cells capable of responding to injury signals, upon which they can migrate, proliferate and differentiate to repair the damaged skeletal muscle

fibres [31]. In 1978, however, Nathanson *et al.* [32] reported that cloned skeletal myoblasts, when grafted onto demineralised bone, could transdifferentiate into cartilage, suggesting the plasticity of the myoblasts, as their differentiation was affected by their microenvironment. Thus, the author's group speculated in the early 1990s that transplantation of skeletal myoblasts into the myocardial environment might induce them to transdifferentiate into cardiomyocytic phenotype. The first experimental data from these studies were published in the journal 'Cell Transplantation' in 1992 [33], which ushered in the concept of cell therapy for myocardial regeneration or 'cellular cardiomyoplasty' [34]. During the following decade, many investigators joined and expanded on these initial observations, confirming that myoblasts implanted within a myocardial scar could survive and differentiate into striated muscle fibres [35-37]. The presence of such fibres had been reported to ameliorate the cardiac function depressed by the presence of a myocardial scar [38]. A Phase I clinical trial was initiated in France in the year 2000 [39], and more recently in the US and elsewhere, as will be discussed further below. There is an ongoing debate among investigators as to whether these myofibres derived from myoblast implantation into the myocardium expressed skeletal myofibre phenotype or cardiomyocytic phenotype [40-42]. However, it recently became known that, contrary to earlier beliefs that myoblasts are all progenitor cells destined to become skeletal muscle fibres, a small fraction of them, known as SP cells, are in fact pluripotent stem cells, possibly of haematopoietic origin [43]. It is, therefore, possible that in 'myoblast transplantation' we could have actually been implanting a mixture of skeletal muscle progenitor cells and SP cells, with the latter being capable of differentiating into a cardiomyocytic phenotype within the myocardial microenvironment. This again points to the difficulty of comparing the outcomes without precise knowledge of the nature of donor cells, resulting in contradictory findings.

### 2.4 Other sources of adult stem cells

Although, to date, most of the cell therapy studies for myocardial regeneration have used skeletal myoblast and bone marrow-derived cells as the donor source, there have been some studies using pro-endothelial cells from the circulating blood for myocardial angiogenesis, as well as the resident stem cells in the liver [3,44] and other tissues for cardiac myogenesis. Further confirmatory studies are needed to evaluate their future potential.

## 3. Physiological roles of marrow-derived adult stem cells

### 3.1 Tissue injury and adult stem cells

The experimental [45-47] and clinical data published in the last several years have revealed the possible pathophysiological roles of marrow-derived adult stem cells. In experimental animal studies, labelled bone marrow adult stem cells are shown to be mobilised and recruited within hours of myocardial infarction, trafficking through the blood circulation to reach the injured myocardium. Homing into the infarct and peri-infarct sites, the cells undergo *in situ* differentiation and express various phenotypes, including those of cardiomyocytes, vascular endothelial and smooth muscle cells and myofibroblasts. It is speculated that these cells participate in several aspects of the healing process known to take place following myocardial infarction, including angiogenesis and development of collateral circulation; maturation of the scar tissue to minimise scar expansion and rupture; and augmentation of peri-infarct cardiomyocytes to modulate the ventricular remodelling process. In this sense, cell therapy using autologous stem cells can be seen as an enhancement of the natural healing process of the myocardium, since obviously, the inherent capacity for healing following tissue injury has its limitations. The analogy here could be that in response to an infection, the phagocytes are mobilised from the bone marrow and the circulating blood to attack the invading microorganisms. Yet the invaders may overcome this defence mechanism and cause sepsis and death, thus therapeutic intervention is often required. The importance of phagocytes in fighting infection is realised only when they are not available, such as in patients with agranulocytosis. Such patients would be quickly overwhelmed by the sepsis without the natural defence provided by the phagocytes and immune system. It could therefore be speculated that in the absence of an innate healing system provided by the adult stem cells, patients who suffer myocardial injury could fare much more poorly, with a worse clinical outcome than is actually encountered. Cell therapy may help by enhancing the healing process, thus improving the outcome.

### 3.2 Recruiting and homing signals

The molecular signals for mobilising bone marrow stem cells and homing of them into the targeted site of myocardial injury are currently under active investigation [48,49]. Inflammatory cytokines released at the injury site may provide the signal(s) for recruitment of marrow stem cells. Miki *et al.* [50] and others [51,52] recently reported that both granulocyte colony stimulating factor (G-CSF) and macrophage colony stimulating factor (M-CSF) improved ventricular function after infarction by mobilising bone marrow cells for tissue repair, while studies by Fujita *et al.* [53] found that G-CSF improved postinfarction heart failure by mobilising bone marrow stem cells, but granulocyte macrophage colony stimulating factor (GM-CSF) had the opposite effect in a mouse myocardial infarction model. Identifying the signalling molecules could have therapeutic potential in itself, since their administration may enhance the mobilisation of existing marrow stem cells of the patients.

The homing signals for stem cells to localise themselves at the injured site are not well understood. In the case of bone marrow cell transplantation to restore damaged bone marrow, various cellular adhesion and chemokine receptors, such as stroma derived factor-1 (SDF-1), are thought to play central

roles in their homing mechanisms [54,55]. Integrins, such as very late antigen (VLA)-4, and other adhesion receptors, such as CD-44, are essential for homing to the bone marrow, presumably through interactions with their ligands on bone marrow endothelium and stroma. Although the molecular species involved at the myocardial infarction site may be different, one speculates that the first step of circulating adult stem cells homing into the injured site may also be related to their interaction with local endothelial cells. These cells trapped within the microvasculature have been seen to leave the vascular space and migrate through the interstitial space toward their destination. It is of interest that Chimenti *et al.* [56] have recently reported that growth factors such as hepatocyte growth factor (HGF) can mobilise resident cardiac primitive cells to migrate and repair the infarcted heart.

### 3.3 Microenvironmental signals for stem cell differentiation

In the author's earlier *in vivo* studies [57], it was noted that upon implantation of marrow stromal cells into the myocardium, the stem cells surrounded by scar tissue appeared to differentiate poorly, while those in direct contact with native cardiomyocytes more readily expressed morphology and phenotypic molecular markers of cardiomyocytes. Such findings led to speculation that direct cell-to-cell contact could be an important signalling mechanism for *in situ* differentiation of the adult stem cells. Recent *in vitro* studies [58-60] provided strong support for this view. Adding a culture medium in which the cardiomyocytes had been cultured then removed, to the dish in which marrow derived adult stem cells were being cultured, did not induce their differentiation. Likewise, co-culturing cardiomyocytes with marrow stem cells but separating them with a filter shield, allowing the passage of macromolecules but preventing direct cell-to-cell contact, also failed to induce stem cell differentiation. Only when the adult stem cells were co-cultured with cardiomyocytes, allowing their direct cell-to-cell contact, could they induce differentiation of stromal cells to express cardiomyocytic phenotype. Such a differentiation signalling mechanism would be useful for the organism to avoid heterotopic tissue formation, such as producing bone or cartilage within the heart muscle, which would be detrimental to the healing process. On the other hand, the precise signalling mechanism by which these stem cells participate in angiogenesis is not fully understood, except for the observation that hypoxia in the culture media or tissue appeared to induce the stem cell expression of angiogenic factors, such as vascular endothelial growth factor (VEGF), which may play a role in their participation in vasculogenesis in the ischaemic tissues [61].

### 3.4 Self-assembly of differentiating adult stem cells

Even after the differentiation of the implanted adult stem cells into various phenotypes of cells constituting the myocardium, if they are arranged in random and not integrated with the native structure of the tissue, they will be functionally ineffective. In the case of neocardiomyocytes, the author's group have shown that contact of such cells with native cardiomyocytes and fibres can induce the formation of gap junctions and eventually lead to the full integration of these cells into the existing cardiac myofibres [62]. Applying the experience gained from tissue engineering using bioreactors [63], in which biomechanical forces are applied to align cellular orientations, the author's group hypothesised that gap junction formation allowed the shear stress generated by contacting native cardiomyofibres to induce self-assembly of neocardiomyocytes, presumably through cell surface mechanoreceptors [64] and reorganisation of the cytoskeletal system. There is evidence that in the formation of blood vessels, the orientations of endothelial [65] and vascular smooth muscle cells [66] are also guided by the pulsatile rheological stresses from within their lumens.

The fact that neocardiomyocytes may assemble using existing cardiac fibres as scaffolds imposes a clinically relevant question when one contemplates cardiomyocyte implantation in heart failure patients. The cardiac dilatation caused by heart failure alters the myofibre orientation such that the ventricle changes its shape from the normal ellipsoidal form into a spherical form, which has been shown to be functionally detrimental and is associated with a poorer prognosis [67]. If the neocardiomyocytes were integrated into the spheroidal ventricular structure by taking the fibre orientation of a diseased heart, presumably they could also be functionally compromised. It has been suggested by Buckberg (pers. comm.) that in such cases, one may adopt the strategy of performing ventricular restoration surgery, which alters the shape of the left ventricle from spherical back to ellipsoidal, prior to the implantation of adult stem cells. It is proposed that with this approach, the neocardiomyocytes may be integrated into an *in vivo* scaffold which is functionally more desirable.

## 4. Preprogramming of adult stem cells *in vitro* prior to therapeutic implantation

The culture expansion of donor stem cells prior to implantation offers the opportunity to intervene and manipulate their fate following implantation, by modulating their gene expressions while under culture [68]. *In vitro* gene therapy to enhance angiogenic factor production, such as for VEGF [69], may induce associated angiogenesis to provide a better blood supply. Addition of 5-azacytidine [70-72] or 5-deoxy-azacytidine [73] may activate genes to commit these cells to a cardiomyocytic lineage, such that their implantation within a myocardial scar may still allow for the generation of cardiomyocytes rather than fibroblasts. Recently, Mangi *et al.* [27] transduced marrow stem cells to overexpress *Akt*, in order to induce resistance to apoptosis in the peri-implantation period. They found that *Akt* gene transfer into marrow stromal cells (MSCs) resulted in a significant decrease in cell death, an increase in regenerated myocardial mass and improvement in cardiac function. Thus, the adult stem cells may not only be used for cell therapy, but also as a

vehicle for gene therapy. An increasing variety of gene therapies may be expected using this targeted delivery strategy.

## 5. Techniques and routes for therapeutic delivery of donor cells

### 5.1 Surgical epicardial injections

Donor cells can be surgically injected directly into the selected myocardial site from the epicardial side. The advantages of the epicardial approach include avoidance of injury to visible epicardial coronary arteries and rapid injection through multiple punctures to reduce the volume of the bolus injected per puncture. The smaller bolus volume facilitates their rapid infiltration within the tissue planes and reduces leakage of implanted cells as a result of the contracting myocardium compressing on the injected bolus. To avoid such leakage, it would also be easy to seal off the puncture holes using various sealants or by suture closure. Loss of donor cells from back flow leakage is important, particularly when cells are implanted into a contracting, rather than an akinetic, segment of the myocardium. Technically, epicardial injection can be easily achieved, either by direct needle injection during sternotomy or thoracotomy, concomitant with cardiac and intrathoracic surgical procedures, with or without cardiopulmonary bypass. In current clinical trials [29,39], most patients had received epicardial cell implants following coronary artery bypass procedure, when a segment of the myocardium was found to be non-bypassable and irreversibly damaged. Alternately, for patients who do not require concomitant surgical intervention, minimally invasive surgical techniques can be utilised for epicardial implantation. The procedure can be guided using an intrathoracic camera, which allows visual identification of the myocardial implant site, as well as the epicardial coronary arteries which need to be avoided.

### 5.2 Catheter-based endocardial injections

Direct intramyocardial implantation of donor cells can also be accomplished through less invasive transvascular cardiac catheters. Various imaging techniques can be used for monitoring the procedure, selecting the site of injection, fixation of the needle tip against the myocardium, and avoidance of ventricular wall perforation and coronary artery damage. Various innovations to achieve these goals, for both a minimally invasive surgical approach as well as an endocardial implantation, are becoming available, and they are being tested clinically for their safety and reliability as well as cost benefits.

### 5.3 Injections via coronary arteries

For hearts with diffuse damage rather than a localised injury such as myocardial infarction, transcoronary injection of adult stem cells has the theoretical advantage in having stem cells diffusely distributed within the damaged left ventricular wall. Such an approach had been shown to be feasible experimentally [74,75], as these cells, which were initially found to be lodged into the coronary microvasculature, were seen to be capable of migrating out of the vascular space and underwent *in situ* differentiation [76,77]. One caveat for this approach is the tendency of marrow stromal cells to aggregate together into a multicellular globule, which poses the risk of obstructing arterioles of significant size, thus precipitating myocardial damage. Experimentally, the author's laboratory have found that a massive dose of cells injected as a bolus into the coronary artery may induce cardiac arrest, so safety issues of both the dosage and physical condition of the cell suspension need to be carefully examined.

### 5.4 Peripheral (intravenous) administration strategy

As discussed earlier, the marrow stromal cells have the ability to traffic through the circulating blood and home into the target area of the myocardial injury, thus systemic administration of marrow stromal cells by intravenous infusion may also be feasible. Again, the danger of cellular aggregation causing them to embolise in the pulmonary vasculature needs to be prevented by adequate dilution of the infusate, as well as the avoidance of a bolus injection in preference for slow intravenous infusion. There has been a report that use of vasodilators may also be helpful [78]. Another important consideration in using the peripheral systemic delivery approach is to understand the nature of the homing signals which allow targeting to the myocardial damaged site. The author's preliminary animal study indicates that such a homing mechanism may be weakened or lost when cells are implanted into a heart which has a chronic stable myocardial scar. This observation is consistent with the hypothesis that the homing signal may be associated with an active inflammatory response at the tissue injury site. In a stable chronic scar, such an inflammatory response would have subsided and as a result the circulating donor cells may not be able to find their targets. Thus, the author's group foresee this approach as being clinically useful, largely for patients who suffer from acute myocardial infarction. Whether this also applies to patients with acute myocarditis has not been examined.

## 6. Functional efficacy: preclinical studies

Using various animal models, the functional efficacy of cell therapy in regional myocardial damage has been studied extensively [25,38,79-81]. The animal species used included mouse, rat, rabbit, pig and sheep, and myocardial lesions were induced by coronary artery occlusions, either by ligation or embolisation, as well as by cryo-injury. As the donor cell source, autologous skeletal myoblasts and, more recently, autologous marrow-derived cells have been used. The functional studies were carried out with various methods, including *in vitro* beating heart Langendorff preparation and analyses of *in vivo* pressure–volume loops obtained with conductance and pressure catheters. Echocardiograpy has been widely used to study the effects of cell therapy on ventricular dimension, ejection fraction, systolic and diastolic functions.

Overall, investigators report that cell implantation within or at the border zone of myocardial infarction ameliorated

depression in systolic pressure and myocardial contractility, improved segmental contractions, reduced cardiac dilatation and in some studies improved diastolic compliance. However, in spite of such favourable functional outcomes in most published reports, the precise mechanisms for them are still being debated, as will be discussed further below. In this regard, the possibility of 'publication bias' also needs to be kept in mind, as negative results are less likely to be submitted and accepted for publication.

## 7. Clinical trials

### 7.1 Autologous myoblast implantations

Autologous skeletal myoblast implantation was initiated in the year 2000 [39] at several institutions in Europe [82,83], mostly by epicardial injections during cardiac surgery, but also by endocardial punctures using catheters. When the concomitant procedures were carried out, such as with coronary artery bypass operations, it was difficult to evaluate the safety or the efficacy because of the confounding factors introduced. One issue of concern is the occurrence of postoperative arrhythmia, which in some patients required implantation of automatic defibrillator devices [83]. Nevertheless, it is difficult to determine whether such arrhythmias were in fact caused by cell implantation, since many such patients are prone to arrhythmia even without cell therapy. There was a news report that a fatality occurred in a European study when the cells were injected via a catheter in the left ventricle. More recently, autologous skeletal myoblast implantation has been initiated in the US, with FDA approval. Positron emission tomography scans indicate that implanted cells within the scar survived for many months after implantation, and echocardiographic data reportedly showed some improvement in segmental contractions [75].

### 7.2 Implantation of autologous marrow-derived cells

Marrow-derived cells have been used as donor cells in scattered clinical trials in Japan, Germany [84] and England. A couple of those clinical implantations employed autologous bone marrow aspirates without cell selection, with the hope of inducing angiogenesis and myogenesis. In a very recent report from the UK [29], 14 patients underwent autologous bone marrow cell implantation into the myocardial scar during coronary artery bypass operations. Again, it was reported that echocardiography showed improved function in the area of the scarred myocardium where the bone marrow cells were injected. The improvement was noted at 6 weeks and maintained at 10 months. The bone marrow cells were aspirated from the sternum of the patient at the time of coronary artery surgery and diluted into plasma before injection. It is of some concern that autologous fresh bone marrow transplant into a myocardial scar in a sheep myocardial infarction model had just been reported at the same meeting and no benefits were found [85]. It is noteworthy that these sheep did not receive concomitant coronary artery operations.

### 7.3 Breaking news and participating biotech companies

The Washington Post reported that ~ 50 patients had received cell therapy for cardiac diseases in several centres by November 2002. There is a multi-centre trial underway in Europe and North America, of 300 patients, sponsored jointly by the Public Assistance Hospital of Paris and Genzyme Biosurgery in Massachusetts. Other companies are also getting ready to sponsor myoblast implant clinical trials, such as Florida-based Bioheart, and Diacrin in Massachusetts, while Osiris Therapeutics in Baltimore is focusing on marrow stromal cell therapy. New clinical trial data have been reported in rapid succession [86-89] in recent months. Thus, the number of patients receiving cell therapy for heart failure can be expected to rise rapidly in the near future.

## 8. The scope and limitation of this review: myocardial angiogenesis and immune tolerance of adult stem cells

As stated in the introduction, this review focuses on translational investigations and early clinical trials, and attempts to evaluate recent developments in this rapidly progressing field. However, a number of important issues are not covered in detail in this review. Myocardial angiogenesis has also been undergoing extensive laboratory and clinical research in recent years. Angiogenic proteins, such as VEGF and basic fibroblast growth factor [90], gene therapy [91] and stem cell therapy [92-94] are all being examined aggressively for patients who cannot benefit from current revascularisation procedures. The importance of myocardial angiogenesis in many heart failure patients, particularly for those who suffer from ischaemic cardiomyopathy, is obvious. Many good reviews are available on this subject and interested readers are encouraged to seek such information, as angiogenesis and myogenesis are often complementary in cardiac cell therapy [95,96].

Another fascinating aspect of adult stem cell research involves the recent reports that marrow-derived stem cells may have a unique immunological capacity to induce tolerance in immunocompetent recipients. A few reports on marrow stromal cell allotransplant [97,98] and xenotransplant [9,99] without immunosuppression have appeared, which, if confirmed by further investigations, could suggest the feasibility of a 'universal donor cell'. In a recent report by the author's group on xenotransplant cardiac chimaera, the new 'Danger Model' theory of Matzinger [100,101] was invoked to try to explain the unexpected findings encountered. The availability of 'universal donor cells' would avoid logistic inconvenience of autotransplant while still eliminating the need for immunosuppression. The use of 'universal donor cells' obtained from young donors may also be valuable for patients of old age whose adult stem cells may be qualitatively or quantitatively compromised. Relevant reports are cited in the references so that readers who wish to further explore these aspects of adult stem cell research may consult them.

## 9. Conclusions

In spite of recent controversy as to whether the phenomenon of cell fusion [14,15], rather than pluripotency, explains the capability of certain adult cells to differentiate into various phenotypes, the existence of adult stem cells is now widely accepted [16,30], although their precise characterisation is still evolving. Skeletal myoblasts and marrow-derived stem and progenitor cells are being studied for their biological and physiological roles in tissue injury, such as in myocardial infarction and heart failure. The use of such cells as donors for cell therapy is currently undergoing vigorous experimental evaluation and early clinical trials have been initiated. Although they are still preliminary, the optimistic data obtained to date are encouraging.

## 10. Expert opinion

It is difficult not to share the excitement of using adult stem cell therapy for the treatment of heart failure in the future. Since ischaemia and loss of cardiomyocytes due to necrosis or apoptosis [102] underlie many cardiac pathologies which lead to heart failure, adult stem cell therapy can be expected to be useful not only in myocardial infarction, a pathological condition so far studied almost exclusively in preclinical and early clinical studies, but also in myocardial injury due to end stage valvular diseases, as well as cardiomyopathies of various aetiologies. It would appear that for heart failure due to diffuse cardiomyopathies, therapeutic cell delivery may not be ideal using the most common technique employed so far – namely, local injection within or at the periphery of myocardial infarcts. Therefore, studies using appropriate models of diffuse cardiomyopathy and heart failure to examine the optimal cell delivery strategies need to be pursued. As mentioned earlier in this review, there is some evidence that the homing signals may be related to the presence of acute inflammatory responses to tissue injury, and this may be lacking in stable infarct or heart failure patients. A better understanding of the pathophysiology involved would allow us to tailor therapeutic approaches to the specific pathological condition and stage of the cardiac lesions.

Many other clinically relevant issues also need to be examined before a sound therapeutic regimen can be designed for wide use. Confusion as to what one calls 'adult stem cells' in a particular cell population makes it difficult to standardise the donor cell preparation, which in turn can cause difficulties in the comparison of data obtained from one study to the other. Sound techniques need to be developed to enable the quantitative study of cells which were retained upon implantation, then survived and differentiated into a specific phenotype. From this, a dose–response relationship, namely the number of cells implanted versus the magnitude of functional improvements achieved, should be obtained in order to know how many cells should be implanted. Many preclinical studies reported so far had shown that implantation of not only stem cells and progenitor cells, but even differentiated smooth muscle cells [103] and fibroblasts within a scar, could improve global and segmental regional contractile functions for which a sound physiological explanation is lacking. How any tissue, even cardiomyofibres, which are surrounded by a scar tissue, can contract in synchrony with the native myocardium in the ventricular wall to improve both global and segmental contractile function, remains obscure [40,104]. Whether the implantation of progenitor cells, which develop into cardiomyocytes within a scar, can in fact replace the scar tissue by promoting apoptosis of the fibroblasts and reduction in the extracellular matrix of the scar, would also be of interest to know [105].

In Phase I clinical trials, the most significant concern is that of safety. In the model of local injection of cells within the scar, arrhythmias could be an issue, since an isolated island of muscle tissue within the myocardium is thought to be a possible arrhythmogenic focus. Although postoperative arrhythmias have not conclusively been shown to be related to cell transplantation, limited early clinical trials already have death and morbidity associated with arrhythmias. In spite of this, adequate cardiac electrophysiological studies have not been performed, which is surely a high priority that should be funded and carried out.

There is a tremendous impetus to proceed rapidly toward clinical trials and wide clinical applications of this exciting therapy. Many cardiac patients who cannot be salvaged with conventional therapy are eagerly waiting for this to become clinically available. Biotech industries are clearly highly motivated to take this from the laboratory to the patients' bed side, and physicians and surgeons share this enthusiasm. Nevertheless, we have to temper this excitement with caution, lest we repeat the tragedies associated with gene therapy in the last few years. An aggressive and hasty attitude leading to errors could set back the progress of this highly promising therapy for years. Continued active preclinical research, addressing the issues discussed above, will help us to select optimal strategies to use adult stem cell therapy for heart failure, which has the potential of benefiting a vast number of patients.

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| Study | Patients, N | Procedure | Follow-up Period | Donor Cell | Complications |
|---|---|---|---|---|---|
| Hamano et al ** | 5 | Myocardial injection during CABG | 1 y | Bone marrow cells | None |
| Strauer et al** | 10 | Intracoronary infusion during PTCA | 3 mo | Bone marrow cells | None |
| Assmus et al** | 20 | Intracoronary infusion during PTCA | 4 mo | Progenito cells | None |
| Menasché et al** | 10 | Myocardial injection during CABG | 10,9 mo | Skeletal myoblasts | 1 death |
| Stamm et al** | 6 | Myocardial injection during CABG | 3-9 mo | Bone marrow cells | 2 patients experienced SVT |
| Pagant et al** | 5 | Myocardial injection during LVAD | 68-191 d | Skeletal myoblasts | 4 patients experienced arrhythmias; 1 LVAD death |
| Tse et al** | 8 | Myocardial injection during catheterization | 3 mo | Bone marrow cells | None |
| Ferin et al** | 14 | Myocardial injection during catheterization | 4 mo | Bone marrow cells | 1 death: no arrhythmias |
| Wollert et al** | 30 | Intracoronary infusion during PTCA | 6 mo | Bone marrow cells | None |
| Brehm et al** | 20 | Intracoronary infusion during PTCA | 3 mo | Bone marrow cells | None |
| Smits et all** | 5 | Myocardial injection during catheterization | 6 mo | Skeletal myoblasts | 1 patient experienced VT |